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(54) Title: METHOD FOR PREVENTING INCREASED IRIDIAL PIGMENTATION DURING PROSTAGLANDIN TREATMENT

(57) Abstract

Anti-inflammatory agents are used to prevent iridial pigmentation during prostaglandin treatment and to manufacture pharmaceuticals for this purpose. A method for preventing iridial pigmentation as well as a method for treating glaucoma is based on the concomitant or simultaneous administration of a prostaglandin or prostaglandin analogue and an anti-inflammatory agent. Both non-steroid and steroid anti-inflammatory agents can be employed according to the invention.

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METHOD FOR PREVENTING INCREASED IRIDIAL PIGMENTATION DURING PROSTAGLANDIN TREATMENT

The present invention is related to a method whereby increased iridial pigmentation which occurs during topical prostaglandin treatment can be prevented, avoided or at least largely reduced. Prostaglandin analogues are commonly used for the treatment of glaucoma, and one of the local side-effects of prostaglandin treatment in the eye is increased iridial pigmentation. The invention also relates to ophthalmic compositions containing medicaments that prevent the development of increased iridial pigmentation during prostaglandin treatment.

Background

Glaucoma is an eye disorder characterised by increased intraocular pressure, excavation of the optic nerve head, and gradual loss of the visual field. An abnormally high intraocular pressure is known to be the most important risk factor for the development of glaucoma. The exact pathophysiological mechanism of open angle glaucoma, however, is still unknown. Unless treated glaucoma may lead to blindness, the course of the disease typically being slow with progressive loss of vision. The intraocular pressure in humans is normally in the range of 12-21 mmHg. At higher pressures e.g. above 21 mmHg there is an increased risk that the eye may be damaged. In one particular form of glaucoma, normal tension glaucoma, damage may, however, occur at intraocular pressure levels that are within the normal physiological range. The opposite situation is also known when the intraocular pressure exceeds the normal range without causing damage to the eye. These cases are referred to as ocular hypertensives. In all these cases it is generally regarded that a reduction of the intraocular pressure is beneficial for the eye.

Glaucoma can be treated by drugs, laser or surgery. Recently, prostaglandin analogues have been introduced for the treatment of glaucoma and such analogues are now being commonly used in many parts of the world. The prostaglandin analogues effectively reduce the pressure in the eye by increasing the drainage of aqueous humor from the eye. Two analogues are currently on the market, namely latanoprost (Xalatan®, Pharmacia & Upjohn Co., USA) and isopropyl unoprostone (Rescula®, Ueno Fine Chemicals, Ltd., Japan), and information on both drugs can be found in the literature (e.g. Stjernschantz and Alm, Curr. Opin. Ophtalmol., 7; 11-17, 1996; and Yamamoto *et al.*, Surv. Opthalmol. 41, Suppl. 2, S99-S103, 1997).

Prostaglandins are endogenous fatty acids usually derived from the precursors eicosatrienoic, eicosatetraenoic and eicosapentaneoic acid through metabolic steps involving oxygenation. The precursors are released from the phospholipids of the cell membrane by lipases, in particular phospholipase A2. The oxygenation of the precursors to the endoperoxide intermediates is catalysed by the cyclo-oxygenase enzyme. There are two cyclo-oxygenase isoenzymes, cyclo-oxygenase-1 (COX-1), and cyclo-oxygenase-2 (COX-2). COX-1 is a constitutive enzyme that continuously generates small amounts of prostaglandins for physiologic purpose, while COX-2 is an inducible enzyme which is typically expressed during inflammation and certain other pathological conditions.

The prostaglandins carry a cyclopentane ring to which two carbon chains attach, the upper usually being called the alpha chain and the lower the omega chain. The prostaglandins are classified in subgroups A, B, C, D, E, F, G, H, I, and J depending on the structure and substituents in the cyclopentane ring as demonstrated in Fig. 1.

The alpha chain is a 7 carbon carboxy-terminated aliphatic chain while the omega chain contains 8 carbons including a terminal methyl group. Subscripts of 1-3 are given depending on the number of double bonds. In prostaglandins with subscript 1 the double bond is situated between carbons 13 and 14 in the omega chain. In prostaglandins with subscript 2 an additional double bond is situated between carbons 5 and 6 in the alpha chain, and prostaglandins with subscript 3 contain a third double bond between carbons 17 and 18 in the omega chain. The molecular structures of latanoprost, isopropyl unoprostone and travaprost (Alcon Inc., USA) are shown in Fig. 2. While currently only two prostaglandin analogues have been introduced on the market for glaucoma treatment it is anticipated that several different prostaglandins analogues will reach the market in the future, and that many if not all of these will cause increased iridial pigmentation as a side-effect. The present invention thus also applies to future prostaglandin analogues that cause increased iridial pigmentation.

Furthermore, recently prostaglandin derivatives called "Novel ocular hypotensive lipids™" (Allergan Inc., USA) in which the carboxylic acid moiety has been substituted with an alcohol or ether group have been presented and may be marketed in the future. Such derivatives are also considered as prostaglandins according to the present invention. Examples of such specific analogues include AGN 190910, AGN 191129 and 192024 (Allergan Inc., USA).

As mentioned above a novel side-effect that is sometimes encountered during topical prostaglandin treatment is increased pigmentation of the iris (Selén *et al.*, Surv. Opthalmol. 41, Suppl. 2; S125-S128, 1997; Wistrand *et al.*, Surv. Opthalmol. 41, Suppl. 2; S129-138, 1997). Both latanoprost (Wistrand *et al.*, J. Glaucoma, 6; 430-432, 1997) and isopropyl unoprostone (Yamamoto and Kitazawa, 1997) have been reported to cause this side-effect during chronic therapy in man. Consequently in some patients the eye colour may turn darker in the direction of brown. While this side-effect apparently has no harmful medical consequences it nevertheless is a disadvantage particularly from a cosmetic point of view in some patients. It would thus be desirable to identify a method whereby the increase of iridial pigmentation during prostaglandin therapy could be avoided.

Summary of the invention

The above problem is solved in an unexpected way, that is through the use of anti-inflammatory drugs and the methods of treatment disclosed in the attached claims. Further embodiments and advantages of the present invention will be evident from the description and examples.

Description

The present inventors have unexpectedly found that latanoprost, used as the free acid, stimulates the production of endogenous prostaglandins, at least PGE_2 and $PGF_{2\alpha}$, in iridial melanocytes, and that this production of endogenous prostaglandins by latanoprost acid can be prevented or markedly reduced by non-steroid anti-inflammatory agents such as indomethacin and NS-398 as well as with the steroid (corticosteroid) anti-inflammatory agent dexamethasone. Since it is known from previous studies that both PGE_2 and $PGF_{2\alpha}$ can elicit increased pigmentation of the iris of monkeys in a similar way as latanoprost (Selén *et al.*, 1997) it is now apparent that the latanoprost effect is mediated by endogenous prostaglandins such as $PGF_{2\alpha}$ and $PGE_{2\alpha}$ since our experiments demonstrate that latanoprost stimulated the formation of these prostaglandins in the iridial melanocytes. Thus it may be possible to prevent the formation of pigment in the iridial melanocytes during chronic latanoprost treatment by concomitant treatment with an anti-inflammatory agent, preferentially a COX-1 or COX-2 inhibitor.

Accordingly, patients that are being treated with either latanoprost or isopropyl unoprostone, and in the future possibly with other prostaglandin derivatives or analogues who are at risk of developing increased iridial pigmentation may be treated simultaneously with an anti-inflammatory agent to prevent increase in iridial pigmentation. Such anti-inflammatory agents include but are not limited to: indomethacin, ibuprofen, diclofenac, etodolac, flurbiprofen, ketorolac, acetosalicylic acid, salicylic acid, salsalate, valeryl salicylate, bismuth subsalicylate, aceto-aminophen, sulindac, aceclofenac, piroxicam, tenoxicam, lornoxicam, meloxicam, naproxen, nabumeton, ketoprofen, azapropazon, fenoprofen, mefenamic acid, oxaprosin, tolmetin, 6-MNA, NS-398, nimesulide, DuP 697, L 745,337, celecoxib, rofecoxib and steroid anti-inflammatory agents such as dexamethasone, prednisolone, methylprednisolone, prednisone, cortisone, hydrocortisone, fluorometholone, triamcinolol, betamethasone, fludrocortisone and deflazacort, and combinations thereof.

The anti-inflammatory agent can be administered locally, e.g. as eye drops, ointments or inserts, or it can be given systemically e.g. orally. Prodrugs, e.g. for enhancing stability or bioavailability of the anti-inflammatory agents are also covered by the present invention. Such prodrugs comprise e.g. sulindac and nabumetone.

According to one embodiment, the present invention defines a method of using *per se* known anti-inflammatory drugs, preferentially so called non-steroid anti-inflammatory agents, for the prevention of increased iridial pigmentation during topical prostaglandin treatment. The method of preventing increased iridial pigmentation during prostaglandin treatment comprises administering the anti-inflammatory drug that prevents endogenous prostaglandin synthesis either directly on the eye or systemically, i.e. orally, once or several times daily, or possibly even with longer intervals.

According to another embodiment, the present invention defines ophthalmologic compositions for administering the anti-inflammatory agents topically on the eye whenever such compositions of the specified compounds have not been disclosed previously. In particular compositions containing both the prostaglandin analogue and the anti-inflammatory agent are novel and highly useful for the patients. The anti-inflammatory agent (and whenever possible together with the prostaglandin analogue) is mixed with an ophthalmologically compatible vehicle known *per se*. The vehicle which may be employed for preparing compositions of this invention comprises aqueous solutions, such as physiological

saline, oil solutions, creams and ointments. The vehicle may furthermore contain ophthalmologically compatible preservatives such as benzalkonium chloride, surfactants e.g. polysorbate 80, liposomes or polymers, e.g. methyl cellulose, polyvinyl alcohol, polyvinyl pyrrolidone and hyaluronic acid; these polymers may be employed to increase the viscosity of the solutions. Inclusion complexes such as cyclodextrins can be used to enhance stability and delivery. Incorporation of the active drugs into soluble and insoluble drug inserts is also possible.

According to a third embodiment, the present invention relates to the preparation of ophthalmologic compositions containing anti-inflammatory agents to be used concomitantly with prostaglandins in the treatment of glaucoma, and in particular compositions that include both the prostaglandin analogue and the anti-inflammatory agent.

The invention is illustrated by means of the following non-limiting examples:

Methods:

Iridial melanocytes were isolated from bovine eyes obtained from a local slaughter house. The melanocytes were cultured in a DMEM, Glutamax/Ham's f-12 culture medium containing 10% fetal calf serum, 1 ng/ml basic human recombinant fibroblast growth factor, 10 ng/ml cholera toxin, 0.1 mM isobutylmethylxanthine (IBMX), 50 µg/ml gentamicin, and 0.25 µg/ml Fungizone. The cells, usually used at passages 2-4, were plated into 48 or 96 well microtiter plates, and grown to confluence. Each well usually contained around 50.000 cells. The test substances, latanoprost, used as the free acid, and the various blocking agents were administered to the cells by adding them to the culture medium. The cells were incubated for 24 hours in the presence of latanoprost and the blocking agents at 37°C in an atmosphere of 95% oxygen and 5% carbon dioxide. For investigating the blocking effect of dexamethasone the cells were preincubated with dexamethasone for 24 hours before the exposure to latanoprost acid and dexamethasone. After the incubation 0.2 ml of the culture medium was aspirated and centrifuged in an Eppendorff centrifuge at 8000 rpm for 5 min. The concentration of PGE_2 and $PGF_{2\alpha}$ in the culture medium was then measured using commercial enzyme immunoassay kits for PGE_2 (Amersham Life Science), and $PGF_{2\alpha}$

(Cayman Chemicals) following appropriate dilutions. The assays were performed according to the manual of the respective commercial enzyme immunoassay kit.

Test substances:

Latanoprost acid was obtained by synthesising according to the method previously described (Resul *et al.*, J. Med. Chem. 36; 243-248, 1993). The acid of latanoprost was used instead of the isopropyl ester because it is not known whether the melanocyte cultures express esterases that can hydrolyse latanoprost to the free acid. In the human eye latanoprost (13,1 4-dihydro- 1 7-phenyl- 18,1 9,20-trinor-$PGF_{2\alpha}$-isopropyl ester)is hydrolysed to the free acid. Indomethacin was obtained from Sigma Chemicals, and was freshly prepared by dissolving in water solution using Na_2CO_3. NS-398 (N-(2Cyclohexyloxy-4-nitrophenyl) methane-sulphonamide) was obtained from Calbiochem-Novabiochem and was dissolved in dimethyl-sulphoxide (DMSO). Dexamethasone was obtained from Sigma Chemical Company and was dissolved in ethanol. The final concentrations of the test substances in the experiments were the following; latanoprost acid 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} M, indomethacin 10^{-5} M, NS 398 10^{-8}, 1 and 10^{-6} M, and dexamethasone 10^{-6} M. The concentrations of the various test substances are generally known to be effective.

Results:

Latanoprost acid caused a release of PGE_2 and $PGF_{2\alpha}$ into the culture medium from the melanocytes in a dose dependent manner (Table I). Maximum effect was seen with a concentration of latanoprost of 10^{-5} M. A near maximum effect was seen with the ten times lower concentration of 10^{-6} M. As can be seen in Table I the melanocytes produced much more PGE_2 than $PGF_{2\alpha}$ when exposed to latanoprost.

Table I.

Effect of latanoprost acid on the production and release of PGE_2 and $PGF_{2\alpha}$ from bovine iridial melanocytes in culture, and blocking effect of indomethacin (10^{-5} M). (Mean ± SEM; ng = nanogram)

Test compound	Concentration (Moles/l)	PGE_2 (ng/well)	$PGF_{2\alpha}$ (ng/well)
Control (vehicle)	0	9.7 ± 2.0	9.4 ± 0.1
Latanoprost acid	10^{-8}	13.5 ± 0 3	---
Latanoprost acid	10^{-7}	---	11.6 ± 1.1
Latanoprost acid	10^{-6}	22.6 ± 1 9	12.1 ± 0 8
Latanoprost acid	10^{-5}	23.7 ± 0.4	12.8 ± 0.8
Latanoprost acid + Indom.	10^{-5}	3.1 ± 0.1	7.9 ± 0.1

Indomethacin, a non-selective inhibitor of the COX- 1 and COX-2 enzymes completely blocked the latanoprost acid induced release of both PGE_2 and $PGF_{2\alpha}$ from the iridial melanocytes (Table I). Likewise the relatively selective COX-2 blocking agent NS-398 at high concentrations (10^{-6} M) markedly blocked the formation of PGE_2, but even at lower concentrations the formation of PGE_2 was partly blocked (Table II). Since the IC_{50} value of NS-398 for blocking the COX-2 enzyme has been reported to be around 10^{-7} M, while the corresponding value for blocking the COX- 1 enzyme was reported to about 10^{-5}M (Patrono *et al.*, Improved non-steroid anti-inflammatory drugs; COX-2 enzyme inhibitors (Eds. Vane, J., et al.) Kluwer Acad. Publ., 121-131, 1996), the results suggest that a large part if not all of the endogenous PGE_2 was produced through the COX-2 enzyme pathway when the cells were exposed to latanoprost acid. As can be seen in Table III dexamethasone, a steroid anti-inflammatory drug that inhibits the phospholipase A2 enzyme through the induction of lipocortin partly blocked the formation of PGE_2 by the melanocytes during exposure to latanoprost acid. Since the anti-inflammatory agents used, either deprive the cells of free arachidonic acid or block its conversion into cyclic endoperoxides the results of the blocking experiments also apply to $PGF_{2\alpha}$, and any other endogenous prostaglandin and thromboxane.

Table II.

Blocking effect of NS-398, a selective cyclo-oxygenase II inhibitor, on latanoprost acid-induced production of endogenous PGE_2 in bovine iridial melanocytes. Latanoprost acid was used at a concentration of 10-5 M (Mean ± SEM; ng = nanogram).

Test compounds	Concentration of blocking agent (Moles/l)	PGE_2 (ng/well)
Vehicle	0	24.5 ± 0.5
Latanoprost acid	0	104.1 ± 0.3
Latanoprost acid + NS-398	10^{-9}	65.5 ± 17 0
Latanoprost acid + NS-398	10^{-8}	32.6 ± 1.2
Latanoprost acid + NS-398	10^{-7}	11.8 ± 1 4
Latanoprost acid + NS-398	10^{-6}	6.8 ± 0.4

(The reason for the larger amount of PGE_2 as compared to Table I was a larger number of cells/well)

Table III. Blocking effect of dexamethasone on latanoprost acid-induced production of PGE_2 in bovine iridial melanocytes. Latanoprost acid was used at a concentration of 10^{-5} M. (Mean ± SEM; ng = nanogram)

Test compounds	Concentration of blocking agent (moles/l)	PGE_2 (ng/well)
Vehicle	0	20.5 ± 2.0
Latanoprost acid	0	43.1 ± 1.2
Latanoprost acid + Dexamethasone	10^{-6}	23.9 ± 1.4

From these experiments it is obvious that latanoprost, a synthetic prostaglandin analogue, causes endogenous formation of PGE_2 and $PGF_{2\alpha}$ in iridial melanocytes, and that the effect can be blocked by the inhibition of the necessary enzymes in the formation of the endogenous prostaglandins. From previous experiments it is clear that both $PGF_{2\alpha}$ and PGE_2 have the capacity to induce increased pigmentation in primates under *in vivo* conditions. It is thus likely that endogenous prostaglandins mediate the melanogenic effect of latanoprost a synthetic prostaglandin analogue.

Accordingly, medicaments that interfere with the prostaglandin synthesis can be predicted to prevent the formation of melanin in the melanocytes. Such medicaments may inhibit either the cyclo-oxygenase 1 or 2 enzymes or the phospholipase A2 or phospholipase C enzymes or any other enzyme necessary in the production of endogenous prostaglandins.

Various non steroid anti-inflammatory agents as well as steroid anti-inflammatory agents have the ability to interfere with the enzymes necessary for prostaglandin synthesis and may therefore be employed according to the present invention. Thus, patients at risk may be treated with anti-inflammatory agents in parallel with prostaglandin treatment used for the reduction of the intraocular pressure in glaucoma therapy.

Experimental part added during the priority year:

Additional experiments have been performed to demonstrate the general validity of the inventive concept, i.e. that anti-inflammatory agents that block the prostaglandin synthesis in melanocytes prevent melanogenesis and thus increased iridial pigmentation during topical prostaglandin treatment of the eye. Topical prostaglandin preparations are used as medicaments for the treatment of glaucoma. Two series of experiments have been performed; 1) to show that not only latanoprost causes increased production of endogenous prostaglandins (e.g. PGE_2) but also other prostaglandins, including analogues that are used or being developed for the treatment of glaucoma ($PGF_{2\alpha}$, fluprostenol and unoprostone acid), and 2) to show that simultaneous treatment of pigment producing cells with indomethacin and latanoprost results in a reduced production of melanin in the cells as compared to latanoprost treatment only demonstrating a true blockade of the melanogenic effect of prostaglandins by anti-inflammatory agents such as indomethacin.

The prostaglandins used in the additional experiments were the following:

$PGF_{2\alpha}$ (prostaglandin $F_{2\alpha}$)
Latanoprost (13,14-dihydro-17-phenyl-18,19,20-trinor-$PGF_{2\alpha}$)
Fluprostenol (16-phenoxy-3-trifluormethyl-18,19,20-trinor-$PGF_{2\alpha}$)
Unoprostone acid (13,14-dihydro-15-keto-$PGF_{2\alpha}$-20-ethyl)

All prostaglandin analogues were used as acids instead of esters which are used clinically for pharmacokinetic reasons.

1. **Increase of the production of endogenous PGE_2 in iridial melanocytes exposed to $PGF_{2\alpha}$, fluprostenol and unoprostone acid.**

These experiments were performed using bovine iridial melanocytes essentially as described in the Methods section above. $PGF_{2\alpha}$ (obtained from Chinoin Chemical and Pharmaceutical Works, Budapest, Hungary), fluprostenol (obtained from Cayman Chemicals, Ann Arbor, Michigan) and unoprostone acid (obtained from Pharmacia & Upjohn, Uppsala, Sweden)

were used at concentrations ranging from 10^{-8} M to 10^{-6} M, and PGE_2 was measured with a radioimmunoassay kit as previously described. The following results were obtained:

Experiment 1.

Prostaglandin	Concentration (Moles/l)	PGE_2 (ng/well
$PGF_{2\alpha}$	0	19
	10^{-5}	37
	10^{-6}	24
	10^{-7}	22
	10^{-8}	12
$PGF_{2\alpha}$ + indomethacin	10^{-5}	17
Fluprostenol	0	6
	10^{-5}	28
	10^{-6}	26
	10^{-7}	24
	10^{-8}	7
Fluprostenol + indomethacin	10^{-5}	0
Unoprostone acid	0	10
	10^{-5}	17
	10^{-6}	12
	10^{-7}	12
	10^{-8}	11
Unoprostone acid + indomethacin	10^{-5}	0

This single experiment clearly demonstrates that the endogenous prostaglandin production in melanocytes is induced by several exogenous prostaglandins and that this thus is a general effect of exogenous prostaglandins. It should also be pointed out that we have utilised PGE_2 as an indicator of endogenous prostaglandin synthesis, but other endogenous prostaglandins such as $PGF_{2\alpha}$, PGD_2, PGI_2 and TxA_2 may also be produced and be of importance in the induction of melanogenesis.

2. Inhibiti n of prostaglandin-induced melanogenesis by indomethacin

The purpose of this experiment was to demonstrate that anti-inflammatory drugs of the NSAID type inhibiting endogenous prostaglandin production have the ability to prevent or reduce melanin formation (melanogenesis) in pigment cells exposed to exogenous prostaglandins. The experiment was performed in the following way:

Cloudman S91 mouse melanoma cells (CCL-53.1) at passages 25-31 were used for the experiment since they produce melanin within a sufficiently short term period to allow measurement of melanogenesis which usually ordinary melanocytes do not. The cells were cultured in a medium containing HAM´s F-10 (82.5%), fetal calf serum (2.5%) and horse serum (15%) in a routine way. The cells were grown to confluence in 80 cm^2 flasks and incubated with the test substances in the flasks. To one flask only control medium was added, to one latanoprost acid at a final concentration of 10^{-6} M and to the third latanoprost acid at a final concentration of 10^{-6} M and freshly prepared indomethacin at a final concentration of 10^{-6} M. The medium including the drugs was replaced after 2 days, and the total incubation period in the presence of the drugs was 4 days. Thereafter the cells were trypsinized and lysated with 1 M NaOH and the melanin content was measured spectrophotometrically at 475 nm with appropriate standards. The melanin content of the cultures were found to be the following:

Experiment 2.

Treatment	Total melanin content	Increase compared to control	Reduction of melanin formation*
Control	163.43 µg	0	0
Latanoprost 10^{-6} M	182.39 µg	18.96 µg	0
Latanoprost 10^{-6} M + Indomethacin 10^{-6} M	175.80 µg	12.37 µg	34.8 %

* compared with latanoprost group

This experiment demonstrates that latanoprost increases formation of melanin in melanoma cells that normally have a high rate of melanin production. When indomethacin is used concomitantly with latanoprost the increase in melanin production was reduced by about 35 %. Although the stimulatory effect of latanoprost of the melanin production is relatively modest it should be kept in mind that the Cloudman S91 melanoma cells have a high rate of spontaneous melanin production and an additional stimulation of the melanin production therefore by the prostaglandin cannot be anticipated to be very marked. Nevertheless the experiment shows that the cells were stimulated to produce more melanin by latanoprost and a considerable part of the stimulation could be blocked by indomethacin, a specific blocker of the cyclo-oxygenase enzyme and thereby endogenous prostaglandin production in the cells.

Thus it is obvious that exogenous prostaglandins e.g. $PGF_{2\alpha}$, latanoprost acid, fluprostenol and unoprostone acid stimulate the formation of endogenous prostaglandins in melanocytes which in turn somehow trigger melanin formation. This prostaglandin induced melanin formation can be blocked by anti-inflammatory agents of the NSAID type.

Finally, the present inventors have also obtained *in vivo* data supporting the mechanism suggested by the *in vitro* data in an ongoing study. In this study 15 cynomolgus monkeys are being treated twice daily with commercially available latanoprost (0.005 %) eye drops (control group), while another 15 cynomolgus monkeys are being treated with commercially available flurbiprofen (0.03 %) eye drops twice daily and the same dose of latanoprost (experimental group). Flurbiprofen is an anti-inflammatory agent which inhibits the cyclo-oxygenase enzyme and thus endogenous prostaglandin formation. After three months treatment 2 of the animals in the control group (2/15), but none of the animals in the experimental group (0/15), had developed clear-cut increase of iridial pigmentation. Thus it appears that blockade of endogenous formation of prostaglandins in the iris prevents increased iridial pigmentation induced by exogenous prostaglandins such as latanoprost. It should be noted that study is scheduled for a longer duration of 12 months.

Although the invention has been described with regard to its preferred embodiments, which constitute the best mode presently known to the inventors, it should be understood that various changes and modifications as would be obvious to one having the ordinary skill in this art may be made without departing from the scope of the invention which is set forth in the claims appended hereto.

Claims

1. Use of an anti-inflammatory agent for the manufacture of a pharmaceutical composition for the prevention of increased iridial pigmentation during prostaglandin treatment of the eye.

2. The use according to claim 1, **characterized** in that the anti-inflammatory agent is of steroid type.

3. The use according to claim 1, **characterized** in that the anti-inflammatory agent is of non-steroid type.

4. The use according to claim 3, **characterized** in that the non-steroid type anti-inflammatory agent is a cyclo-oxygenase inhibitor.

5. The use according to claim 3, **characterized** in that the non-steroid type anti-inflammatory agent is a cyclo-oxygenase-2 inhibitor.

6. The use according to claim 3, **characterized** in that the non-steroid type anti-inflammatory agent is a cyclo-oxygenase inhibitor chosen from the following group: indomethacin, sulindac, etodolac, diclofenac, ketorolac, aceclophenac, piroxicam, tenoxicam, lornoxicam, meloxicam, fenoprofen, ibuprofen, naproxen, ketoprofen, flurbiprofen, nabumeton, azapropazon, mefenamic acid, oxaprosin, tolmetin, acetylsalicylic acid, salicylic acid, salsalate, valeryl salicylate, bismuth subsalicylate, acetoaminophen, 6-MNA, ninesulide, DuP 697, L 745,337, NS-398, celecoxib, rofecoxib and a combination thereof.

7. The use according to claim 3, **characterized** in that the non-steroid type anti-inflammatory agent is a cyclo-oxygenase inhibitor chosen from the following group: meloxicam, nabumeton, NS-398, DuP 697, L 745,337, celecoxib, rofecoxib and a combination thereof.

8. The use according to claim 2, **characterized** in that the steroid type anti-inflammatory agent is chosen from a group of steroid anti-inflammatory agents comprising dexamethasone, prednisolone, methylprednisolone, prednisone, cortisone, hydrocortisone, fluorometholone, triamcinolol, betametasone, fludrocortisone, deflazacort and a combination thereof.

9. Use of an anti-inflammatory agent in combination with a prostaglandin for the manufacture of a pharmaceutical composition for the treatment of glaucoma.

10. The use according to claim 9, **characterized** in that the anti-inflammatory agent is of steroid type.

11. The use according to claim 9, **characterized** in that the anti-inflammatory agent is of non-steroid type.

12. The use according to claim 11, **characterized** in that the non-steroid type anti-inflammatory agent is a cyclo-oxygenase inhibitor.

13. The use according to claim 11, **characterized** in that the non-steroid type anti-inflammatory agent is a cyclo-oxygenase-2 inhibitor.

14. The use according to claim 11, **characterized** in that the non-steroid type anti-inflammatory agent is a cyclo-oxygenase inhibitor chosen from the following group: indomethacin, sulindac, etodolac, diclofenac, ketorolac, aceclophenac, piroxicam, tenoxicam, lornoxicam, meloxicam, fenoprofen, ibuprofen, naproxen, ketoprofen, flurbiprofen, nabumeton, azapropazon, mefenamic acid, oxaprosin, tolmetin, acetylsalicylic acid, salicylic acid, salsalate, valeryl salicylate, bismuth subsalicylate, acetoaminophen, 6-MNA, ninesulide, DuP 697, L 745,337, NS-398, celecoxib, rofecoxib and a combination thereof.

15. The use according to claim 11, **characterized** in that the non-steroid type anti-inflammatory agent is a cyclo-oxygenase inhibitor chosen from the following group: meloxicam, nabumeton, NS-398, DuP 697, L 745,337, celecoxib, rofecoxib and a combination thereof.

16. The use according to claim 10, **characterized** in that the steroid type anti-inflammatory agent is chosen from a group of steroid anti-inflammatory agents comprising dexamethasone, prednisolone, methylprednisolone, prednisone, cortisone, hydrocortisone, fluorometholone, triamcinolol, betametasone, fludrocortisone, deflazacort and a combination thereof.

17. Method for prevention of increased iridial pigmentation during prostaglandin treatment of the eye, **characterized** in that an anti-inflammatory agent is administered to the eye during the prostaglandin treatment.

18. The method according to claim 17, **characterized** in that the anti-inflammatory agent is of steroid type.

19. The method according to claim 17, **characterized** in that the anti-inflammatory agent is of non-steroid type.

20. The method according to claim 19, **characterized** in that the non-steroid type anti-inflammatory agent is a cyclo-oxygenase inhibitor.

21. The method according to claim 19, **characterized** in that the non-steroid type anti-inflammatory agent is a cyclo-oxygenase-2 inhibitor.

22. The method according to claim 19, **characterized** in that the non-steroid type anti-inflammatory agent is a cyclo-oxygenase inhibitor chosen from the following group: indomethacin, sulindac, etodolac, diclofenac, ketorolac, aceclophenac, piroxicam, tenoxicam,

lornoxicam, meloxicam, fenoprofen, ibuprofen, naproxen, ketoprofen, flurbiprofen, nabumeton, azapropazon, mefenamic acid, oxaprosin, tolmetin, acetylsalicylic acid, salicylic acid, salsalate, valeryl salicylate, bismuth subsalicylate, acetoaminophen, 6-MNA, ninesulide, DuP 697, L 745,337, NS-398, celecoxib, rofecoxib and a combination thereof.

23. The method according to claim 19, **characterized** in that the non-steroid type anti-inflammatory agent is a cyclo-oxygenase inhibitor chosen from the following group: meloxicam, nabumeton, NS-398, DuP 697, L 745,337, celecoxib, rofecoxib and a combination thereof.

24. The method according to claim 18, **characterized** in that the steroid type anti-inflammatory agent is chosen from a group of steroid anti-inflammatory agents comprising dexamethasone, prednisolone, methylprednisolone, prednisone, cortisone, hydrocortisone, fluorometholone, triamcinolol, betametasone, fludrocortisone, deflazacort and a combination thereof.

25. The method according to any one of claims 17 – 24, **characterized** in that the prostaglandin is a prostaglandin analogue.

26. The method according to any one of claims 17 – 24, **characterized** in that the prostaglandin is latanoprost.

27. The method according to any one of claims 17 – 24, **characterized** in that the prostaglandin is isopropyl unoprostone.

28. The method according to any one of claims 17 – 24, **characterized** in that the prostaglandin is travaprost.

29. The method according to any one of claims 17 – 24, **characterized** in that the prostaglandin is any one of AGN 190910, AGN 191129 or AGN 192024.

30. Method for the treatment of glaucoma, **characterized** in that that an anti-inflammatory agent is administered to the eye together with a prostaglandin.

31. The method according to claim 30, **characterized** in that the anti-inflammatory agent is of steroid type.

32. The method according to claim 30, **characterized** in that the anti-inflammatory agent is of non-steroid type.

33. The method according to claim 32, **characterized** in that the non-steroid type anti-inflammatory agent is a cyclo-oxygenase inhibitor.

34. The method according to claim 32, **characterized** in that the non-steroid type anti-inflammatory agent is a cyclo-oxygenase-2 inhibitor.

35. The method according to claim 32, **characterized** in that the non-steroid type anti-inflammatory agent is a cyclo-oxygenase inhibitor chosen from the following group: indomethacin, sulindac, etodolac, diclofenac, ketorolac, aceclophenac, piroxicam, tenoxicam, lornoxicam, meloxicam, fenoprofen, ibuprofen, naproxen, ketoprofen, flurbiprofen, nabumeton, azapropazon, mefenamic acid, oxaprosin, tolmetin, acetylsalicylic acid, salicylic acid, salsalate, valeryl salicylate, bismuth subsalicylate, acetoaminophen, 6-MNA, ninesulide, DuP 697, L 745,337, NS-398, celecoxib, rofecoxib and a combination thereof.

36. The method according to claim 32, **characterized** in that the non-steroid type anti-inflammatory agent is a cyclo-oxygenase inhibitor chosen from the following group: meloxicam, nabumeton, NS-398, DuP 697, L 745,337, celecoxib, rofecoxib and a combination thereof.

37. The method according to claim 31, **characterized** in that the steroid type anti-inflammatory agent is chosen from a group of steroid anti-inflammatory agents comprising dexamethasone, prednisolone, methylprednisolone, prednisone, cortisone, hydrocortisone, fluorometholone, triamcinolol, betametasone, fludrocortisone, deflazacort and a combination thereof.

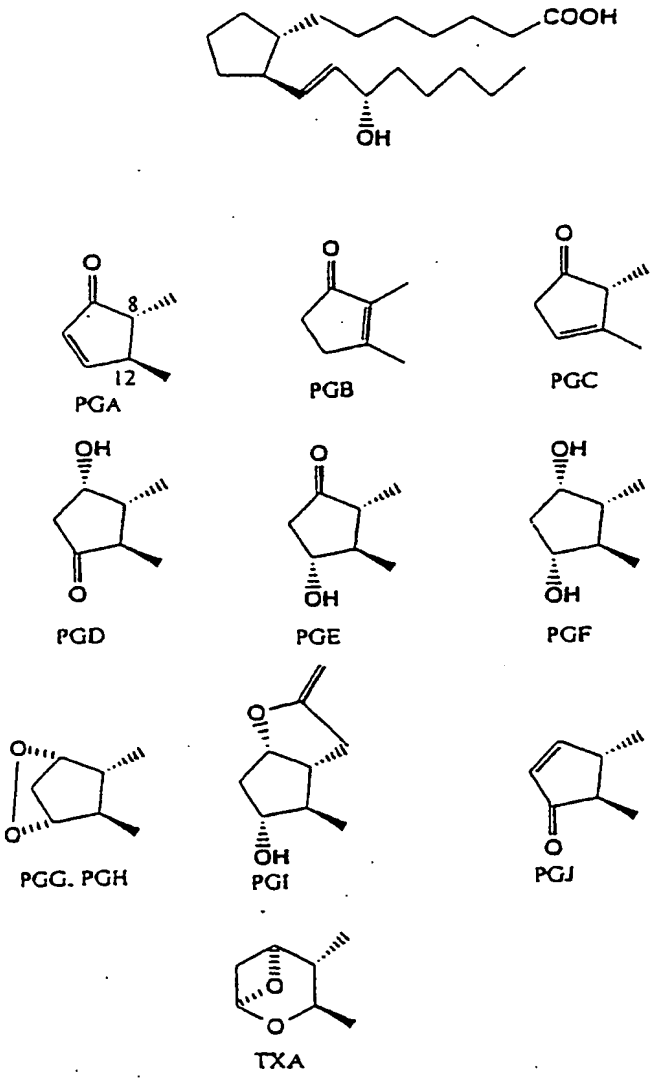
38. The method according to any one of claims 30 – 37, **characterized** in that the prostaglandin is a prostaglandin analogue.

39. The method according to any one of claims 30 – 37, **characterized** in that the prostaglandin is latanoprost.

40. The method according to any one of claims 30 – 37, **characterized** in that the prostaglandin is isopropyl unoprostone.

41. The method according to any one of claims 30 – 37, **characterized** in that the prostaglandin is travaprost.

42. The method according to any one of claims 30 – 37, **characterized** in that the prostaglandin is any one of AGN 190910, AGN 191129 and AGN 192024.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PGA
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PGB
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PGC
OH
O
PGD
O
OH
PGE
OH
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O
PGG. PGH
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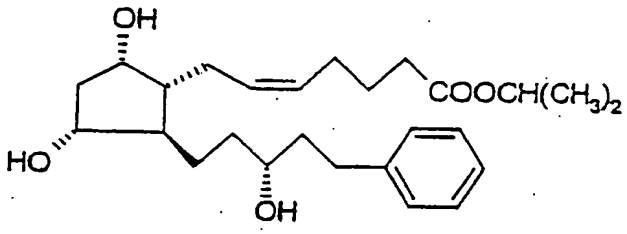
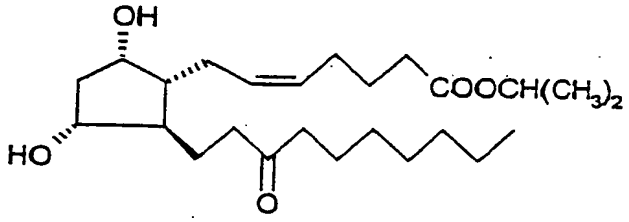
OH
PGI
O
O
PGJ
O
O
TXA

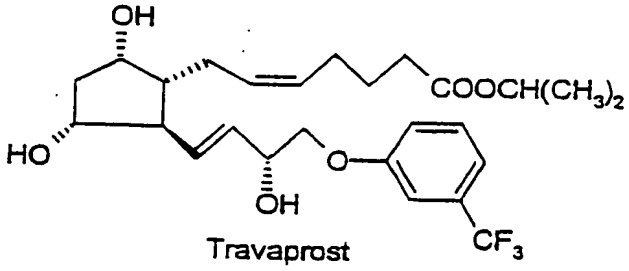
Fig. 1



Latanoprost
Xalatan(R)



Isopropyl unoprostone
Rescula(R)



Travaprost

Fig. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 99/01993

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A61K 31/18, A61K 31/40, A61K 31/12, A61K 31/557, A61K 45/00, A61P 27/06
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Exp. Eye Res., Volume 63, 1996, Sardar Y.K. Yousufzai et al, "Prostaglandin F2alpha and its Analogs Induce Release of Endogenous Prostaglandins in Iris and Ciliary Muscles Isolated from Cat and Other Mammalian Species", page 305 - page 310, page 308 - page 309	1-8,17-29
X	--	9-16,30-42
A	Patent Abstracts of Japan, Vol 3,No 3, C-33 abstract of JP 53-127832 A (Teijin K.K.), 11 August 1978 (11.08.78)	1-42
	--	
A	GB 2135881 A (FARMITALIA CARLO ERBA SPA), 12 Sept 1984 (12.09.84)	1-42
	--	

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" late. document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
31 January 2000	25 -02- 2000
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Gerd Strandell/Els Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 99/01993

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0448274 A2 (E.R. SQUIBB & SONS, INC.), 25 Sept 1991 (25.09.91) --	1-42
A	WO 9520567 A1 (UNIVERSITY OF KENTUCKY RESEARCH FOUNDATION), 3 August 1995 (03.08.95), claims 11, 12 --	1-42
A	EP 0268388 A1 (GLAXO GROUP LIMITED), 25 May 1988 (25.05.88) --	1-42
A	WO 9116895 A1 (G.D. SEARLE & CO.), 14 November 1991 (14.11.91) --	1-42
A	WO 9116886 A1 (G.D. SEARLE & CO.), 14 November 1991 (14.11.91) --	1-42
P,A	WO 9902165 A1 (PHARMACIA & UPJOHN AB), 21 January 1999 (21.01.99) --	1-42

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 99/01993

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 17-42
because they relate to subject matter not required to be searched by this Authority, namely:

see next page

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application. as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 99/01993

Claims 17-42 relate to methods of treatment of the human or animal body by surgery or by therapy. See PCT, Rule 39.1(iv). Nevertheless, a search has been executed for these claims. The search has been based on the allleged effects of the compounds/compositions.

INTERNATIONAL SEARCH REPORT

Information on patent family members

02/12/99

International application No.
PCT/SE 99/01993

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 2135881 A	12/09/84	BE 899033 A	29/08/84
		DE 3404209 A	06/09/84
		IT 1174481 B	01/07/87
		IT 8419388 D	00/00/00
		JP 59164719 A	17/09/84
EP 0448274 A2	25/09/91	AU 647079 B	17/03/94
		AU 7199891 A	19/09/91
		CA 2037561 A	20/09/91
		IL 97513 A	14/05/96
		JP 8026990 A	30/01/96
		US 5312818 A	17/05/94
WO 9520567 A1	03/08/95	AU 705226 B	20/05/99
		AU 6254594 A	15/08/95
		CA 2182228 A	03/08/95
		EP 0740650 A	06/11/96
		JP 9509151 T	16/09/97
EP 0268388 A1	25/05/88	SE 0268388 T3	
		AT 78401 T	15/08/92
		AU 606082 B	31/01/91
		AU 8001587 A	28/04/88
		CA 1307739 A	22/09/92
		DE 3780577 A	27/08/92
		DK 550887 A	23/04/88
		ES 2042580 T	16/12/93
		GB 2197787 A,B	02/06/88
		GR 3005791 T	07/06/93
		JP 63115828 A	20/05/88
		NZ 222261 A	29/01/91
		US 4855293 A	08/08/89

INTERNATIONAL SEARCH REPORT

Information on patent family members

02/12/99

International application No.
PCT/SE 99/01993

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9116895 A1	14/11/91	AT 121625 T	15/05/95
		AU 7876491 A	27/11/91
		CA 2082944 A,C	04/11/91
		DE 69109286 D,T	28/09/95
		DK 527887 T	03/07/95
		EP 0527887 A,B	24/02/93
		SE 0527887 T3	
		ES 2071312 T	16/06/95
		GR 3015939 T	31/07/95
		IE 68596 B	26/06/96
		IL 98033 A	31/07/95
		NZ 238024 A	26/08/93
		PT 97562 A,B	28/02/92
		US 5601843 A	11/02/97
		US 5698225 A	16/12/97
WO 9116886 A1	14/11/91	AU 8089091 A	27/11/91
		IE 62050 B	14/12/94
		IL 98034 A	31/10/96
		NZ 238023 A	26/08/92
		PT 97550 A,B	28/02/92
		US 5213807 A	25/05/93
WO 9902165 A1	21/01/99	AU 8368398 A	08/02/99
		SE 9702706 D	00/00/00